REMARKS

Claim 1 is pending in the instant application. Claim 1 has been rejected. Reconsideration is respectfully requested in light of the following remarks.

I. Amendment to Specification

In accordance with the Examiner's request, Applicants have amended the first line of the specification to delete the phrase in the priority claim regarding incorporation by reference.

II. Rejection of Claims 1 under 35 U.S.C. § 112, first paragraph

Claim 1 has been rejected under 35 U.S.C. § 112, first paragraph, as failing to comply with the enablement requirement. The Examiner has acknowledged the claim to be enabled for a method of detecting the presence of cancer of the stomach or small intestine by detection of mRNA levels in a sample of stomach or small intestine with an increase in measured levels versus normal matched control samples. The Examiner suggests, however, that the specification is not enabled for detection of SEQ ID NO:2 in the sample or detection in just any tissue.

Accordingly, in an earnest effort to advance the prosecution of this case, Applicants are providing herewith a copy of a poster presented at Cambridge Healthtech Institute's Eleventh

Annual Molecular Medicine Tri-Conference and Exposition on March 23-26, 2004 at the Moscone Center in San Francisco confirming that mRNA expression levels of Cln101 (referred to as CC2 in the instant specification) correlate with protein (SEQ ID NO:2) expression levels and that Cln101 or CC2 protein expression was detectable not only in the cancer tissues but in other samples, and in particular serum. In addition to a copy of the complete poster presentation, Applicants are providing herewith an enlarged copy of the graph from the poster with confirming evidence of Cln101 (CC2) protein expression in cancer serum samples.

This poster presentation confirms teachings in the instant specification at page 4, lines 1-16, page 7, line 19 through page 8, line 3, page 11, line 15, through page 12, line 19, and page 13, line 29, through page 14, line 12 regarding expression levels of SEQ ID NO:2 being a diagnostic marker for cancer and teachings in the instant specification at page 14, lines 13-20 that CC2 is detectable in cells, tissues and bodily fluids.

Thus, since Applicants have now provided evidence of the protein's expression including the correlation to a diseases state, one of skill in the art can predictably use the polypeptides in a diagnostic setting in accordance with the

teachings of the specification without any undue experimentation.

Withdrawal of this rejection under 35 U.S.C. § 112, first paragraph is therefore respectfully requested.

III. Conclusion

Applicants believe that the foregoing comprises a full and complete response to the Office Action of record. Accordingly, favorable reconsideration and subsequent allowance of the pending claims is earnestly solicited.

Respectfully submitted,

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Date: **July 28, 2004**

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Identification and Validation of Human Serum Biomarkers Using a Genomic Approach

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Abstract

Using comprehensive mining of cDNA databases and subtractive libraries we identified genes differentially expressed in cancer. These genes are prioritized based on expression profiling using custom microarrays and quantitative RT-PCR, and a variety of bioinformatics tools. We have also implemented a proprietary database for prediction of alternatively spliced transcripts to identify disease-associated, alternative transcripts encoding proteins from genes of interest. We select genes that are up-regulated in cancer that code for secreted proteins and then obtain full-length cDNA clones to produce recombinant proteins to generate mouse monoclonal antibodies. We use these reagents to characterize the biochemistry and cell biology of the proteins and then develop an ELISA assay to analyze the protein expression of these candidate biomarkers in diseased human serum samples and serum samples from normal controls. We will present the identification and validation of Regenerating Protein IV and examples of its potential clinical utility as a cancer biomarker.

Introduction

- Using cDNA database mining strategies, Cln101 encodes a secreted protein, also known as Human Regenerating protein IV (Reg IV) was identified as a colon specific gene that is up-regulated in colon cancer. Reg IV belongs to the Regenerating (Reg) family of proteins characterized by a calcium dependent carbohydrate binding lectin domain (1). Reg proteins have been shown to be involved in regenerative processes following gastrointestinal mucosal response to injury. Others described the over-expression of RegIV mRNA in colorectal carcinomas (2).
- Using a proprietary database of alternative spliced transcripts we searched for alternative transcripts forms (ATF) differentially expressed in cancer. We identified one target of interest, Cln101-V1.
- Real Time Quantitative PCR with Taqman™ fluorescent probe assays were performed in 200 human tissue samples to evaluate the mRNA expression profiles of Cln101 and Cln101-V1 in different anatomical sites and disease states.
- Secretion assays, soft agar assays, and de-glycosylation assays were performed on cell lines over-expressing Cln101 and Cln101-V1.
- A specific sandwich ELISA assay was used to determine levels of Cln101 in serum samples from cancer patients and normal controls.

Methods & Materials

Quantitative Real-time PCR

Over 200 RNA samples from 19 unrelated tissues types were analyzed using real-time PCR (ABI Prism 7900 Sequence detection system, PE Applied Biosystems). Matching samples, cancer and normal adjacent tissue from the same individual. Gene expression was normalized using adenosine triphosphate synthase 6 (ATPsy6).

Cln101 ELISA

Recombinant Cln101 protein (17 kD) was used to develop mouse monoclonal antibodies (mAbs) specific for the protein. A sequential sandwich ELISA for Cln101 was developed using two Cln101-specific mAbs. High binding polystyrene plates (Corning Life Sciences MA) were coated overnight at 4°C with 0.1 ug/well of A46.1 mAb. Twenty uL of serum samples were used in the assay together with 20uL of Cln101 standards at concentrations of 12.8, 6.4, 1.6, 0.4, 0.1 and 0 ng/mL. Biotinylated A8.1 mAb, followed by streptavidin-alkaline phosphatase, and colorimetric signal generation using pNPP was used for detection.

Soft Agar Assay

We produced AP controls, Cln101, and Cln101-V1 retrovirus using virus packaging cells. We infected NIH3T3 target cells and selected with G418. Trypsinized cells were suspended in 0.4 % agar, 10% FBS in Iscove's medium and applied in a 5 ml final volume on top of the solidified base layer. Three different viable cell numbers, 100, 104 and 5X103 cells, were seeded in agar per 6cm2 well in duplicate. A final 2 ml layer consisting of 0.8 % agar, 10% FBS in Iscove's medium was applied on top of the solidified cell layer. The agar plates were then incubated in a humidified 37OC incubator with 5% CO2 for approximately 2 weeks before colonies appeared. The soft agar was maintained by weekly feedings with growth medium. Colonies were counted between 2 and 4 weeks.

Secretion Assay

To determine if a protein is secreted from cells a secretion assay is performed. A pcDNA3.1 clone containing the gene transcript which encodes the protein is transfected into 293T cells using the Superfect transfection reagent (Qiagen, Valencia CA). Transfected cells are incubated for 28 hours before the media is collected and immediately spun down to remove any detached cells. The adherent cells are solubilized with lysis buffer (1% NP40, 10mM sodium phosphate pH7.0, and 0.15M NaCl). The lysed cells are collected and spun down and the supernatant extracted as cell lysate. Western immunoblot is carried out onto a PVDF membrane (Invitrogen, Carlsbad CA). The blot is incubated with a polyclonal primary antibody which binds to the tagged protein. The blot is developed with the ECL Plus chemiluminescent detection reagent (Amersham BioSciences, Piscataway NJ).

Summary and Conclusions

- Both Cln101 and Cln101-V1 show high mRNA expression in tissues from the gastrointestinal tract and overexpression in cancer samples
- Recombinant Cln101 is secreted to the media, while recombinant Cln101-V1 is not
- Recombinant Cln101-V1 is glycosylated, while recombinant Cln101 is not
- Recombinant Cln101-V1 promotes cell proliferation in a soft agar assay, while recombinant Cln101 does not
- A sensitive ELISA for Cln101 was developed and used to test Cln101 levels in serum
- Analysis of 38 normal and 128 cancer serum samples shows that the Cln101 is detectable in serum and is significantly elevated in cancer serum compared to normal controls
- These data suggest that Cln101 could be a useful serum marker for detection of many different cancers

Discovery and Progression

Cancer Lead Discovery and Progression

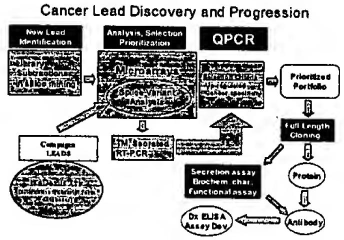
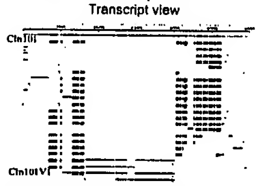


Figure. 1 Cancer lead discovery and validation of transcripts differentially expressed in human cancer

Public and proprietary mRNA and EST databases were used to identify differentially expressed genes. Microarray analysis and a proprietary database of alternative spliced transcripts generated by the Compugen's LEADS platform were used to select and prioritize alternative spliced transcripts differentially expressed in cancer. We identified a total of approximately 700 up-regulated genes containing approximately 1600 alternative transcript forms (ATFs). Among these ATFs, we selected approximately 400 encoding proteins with putative secretion signal (Signal P) or transmembrane domains for further analysis. We performed RT-PCR validation using primers designed to amplify unique sequences for each alternative splice variant. Sequence-validated transcripts were then subjected to real-time quantitative RT-PCR (QPCR) analysis to determine the relative expression level of each transcript among various cancer types. We cloned the genes of the leads prioritized by this process, expressed the proteins and developed specific monoclonal antibodies. We then performed experiments to biochemically characterize the leads and developed specific ELISA assays to detect the proteins in serum. Lastly, we determine if differential levels of the protein in cancer patients could have a specific clinical utility.

Alternative Splicing

Splice variant analysis of Cln101
Transcript view



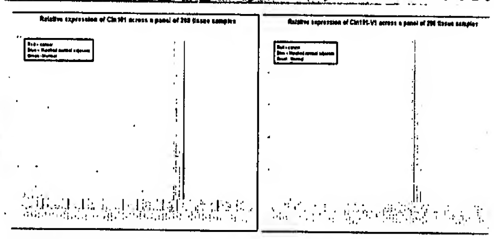
- The Transcripts view displays the consensus sequence for the selected gene, its transcripts, SNPs, proteins, SAGE tags, and predicted proteins.

References

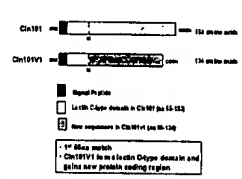
1. Hartupee et. al., 2001., Biochim. Biophys. Acta 1518 (3), 287-293

2. Violette et. al., 2003., Int. J. Cancer 103, 185-193

Cln101/Cln101-V1 mRNA Profiles and Protein Comparison

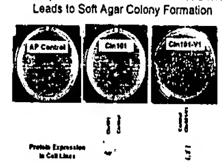


Comparison of Cln101 and Cln101V1 proteins



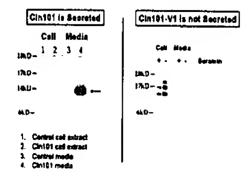
Functional Validation and Biochemistry

Overexpression of Cln101-V1 but not Cln101 Leads to Soft Agar Colony Formation



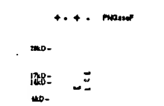
Western immunoblot with antibody against tagged protein

Secretion of Cln101 and Cln101-V1



Western immunoblot with antibody against the tagged protein

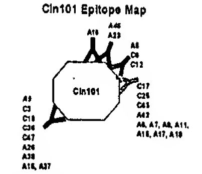
Cln101-V1 but not Cln101 is N-Glycosylated



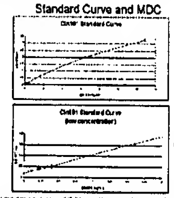
Western immunoblot with antibody against tagged protein

Cln101 ELISA Standard Curve and Serum Expression

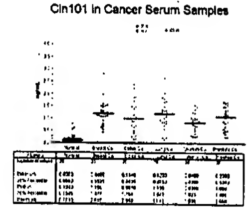
Cln101 Epitope Map



Standard Curve and MDC



Cln101 in Cancer Serum Samples



The figure shows levels of Cln101 in normal and cancer serum. Thirty five serum samples from normal donors, and 25 serum samples from patients with 5 different cancer type were tested with Cln101 ELISA. The data shows that Cln101 is detectable in low levels in serum and that the serum Cln101 levels are significantly higher in cancer patients when compared to normal controls.

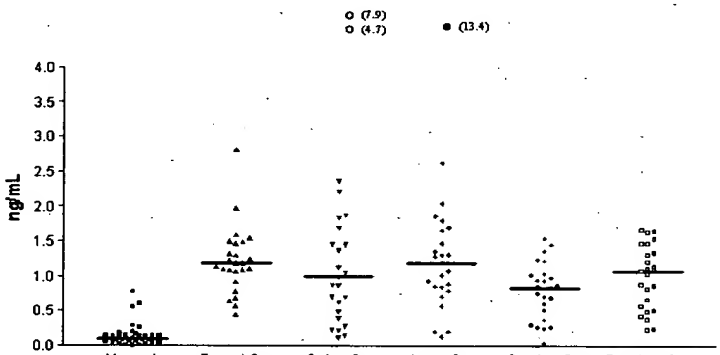
Acknowledgements

We thank Hui-Mei Chen for obtaining the Full-Length clones used in this study. We also thank the Protein Science and Antibody groups for the generation of key reagents.

Western immunoblot with antibody against tagged protein

Expression

Cln101 in Cancer Serum Samples



X Labels	Normal	Breast Ca	Colon Ca	Lung Ca	Ovarian Ca	Prostate Ca
Number of values	35	25	25	25	25	25
Minimum	0.0070	0.4460	0.1140	0.1290	0.0400	0.2300
25% Percentile	0.0550	0.9925	0.4435	0.8150	0.4380	0.5340
Median	0.1000	1.190	0.9870	1.195	0.8310	1.080
75% Percentile	0.1565	1.477	1.764	1.671	1.023	1.401
Maximum	0.7780	2.815	7.953	13.41	1.536	1.668

The figure shows levels of Cln101 in normal and cancer serum. Thirty five serum samples from normal donors, and 25 serum samples from patients with 5 different cancer type were tested with Cln101 ELISA. The data shows that Cln101 is detectable in low levels in serum and that the serum Cln101 levels are significantly higher in cancer patients when compared to normal controls.